FUNCTIONAL MICROSTIMULATION OF THE LUMBOSACRAL SPINAL CORD

Contract NIH-NINDS-No1-NS-2-2342

Quarterly Progress Report #5
Period covered: 1 December 2002 to 28 February 2003

Submitted to:
Neural Prosthesis Program
National Institute of Neurological Disorders and Stroke
National Institute of Health

by:
Rehabilitation Neuroscience Group
Center for Neuroscience/Dept. of Physiology
University of Alberta, Edmonton AB, T6G 2S2 CANADA

Principal Investigator: Arthur Prochazka, Ph.D.[1]
Co-Investigators: Vivian Mushahwar, Ph.D.[1]
John Downie Ph.D.[2]
Susan Shefchyk Ph.D.[3]
Robert Gaunt, B.Eng.[1]

[1] *Center for Neuroscience, University of Alberta, Edmonton, Canada*
[2] *Dept. of Pharmacology, Dalhousie University, Halifax, Canada*
[3] *Dept. of Physiology, University of Manitoba, Winnipeg, Canada*

ABSTRACT

The main aim of this contract is to test the idea that intraspinal microstimulation (ISMS) can be used selectively to excite neurons that activate the bladder detrusor muscle while simultaneously stimulating interneurons which inhibit motoneurons of the external urethral sphincter (EUS). If this reciprocal action works well enough to produce bladder voiding after spinal-cord-injury (SCI), it could form the basis of a neuroprosthesis that would restore bladder control without the need for transection of sensory nerve roots of the spinal cord (dorsal rhizotomies).

In this quarter of operation the following was achieved:

1) First comparison of ISMS-evoked bladder contractions before and after spinal transection (T10 complete) in the intact cat.

2) The time course of bladder pressure responses evoked by ISMS and the maximal pressures achieved were similar before and after spinalization.

3) ISMS elicited voiding in both states, however after spinalization voiding was less complete. This is in line with the possibility raised in our last report that the voiding responses elicited by ISMS in the normal cat may have included triggered responses mediated by supraspinal centres.

4) ISMS sufficient to elicit voiding did not elicit aversive reactions either before or after spinalization. A lack of discomfort during stimulation is an important factor in relation to the possible clinical application of this approach in people with incomplete SCI and preserved sacral sensation.

5) Simultaneous bladder and intraurethral pressure measurements were made in the spinalized cat. Though reciprocal pressure changes were obtained with ISMS in some trials, the more frequent observation was co-activation of detrusor and urethra. Most microwires targeting the commissural region inhibiting sphincter motoneurons did not elicit reductions in intraurethral pressure.

PROGRESS IN THIS QUARTER

METHODS

Implantation of ISMS arrays and bladder catheters

Experiments in this quarter were performed on four male adult cats chronically implanted with ISMS arrays and bladder catheters (Mick 01Oct02, Perry 15Jan03, Pascal 27Jan03, Voodoo17Feb03). For details of the implantation procedures, please refer to Quarterly Report #4. In addition, spinal transection was performed in two of the animals and measurements of bladder and intraurethral pressures evoked by ISMS were made before and after spinalization.

Bladder catheters

As reported previously, the cat implanted with the catheter shown in Fig. 1A had a low tolerance to bladder filling (threshold for voluntary voiding 8-11 ml). We suspected the catheter might have caused irritation to the internal bladder wall. Immediately after spinalization (see below), the urge incontinence completely disappeared. In the weeks since spinalization, urine has been extracted twice daily via the indwelling catheter, with volumes in the range 25 to 47ml. Little if any spontaneous voiding has been observed. This supports the idea that the urge incontinence was due to bladder irritation triggering supraspinal micturition responses. Catheter B has not caused urge incontinence in the three recipients (Perry, Pascal, Voodoo), before or after spinal transection (Pascal). This design will therefore be used in all future implants.

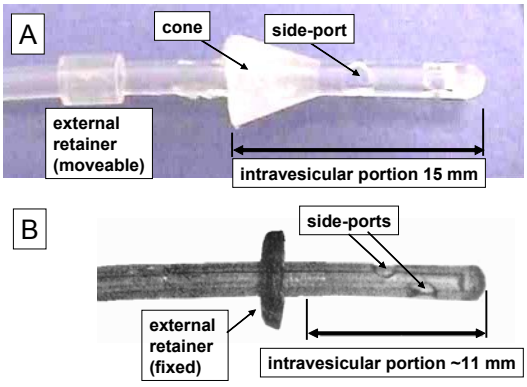

Figure 1. A) Intravesicular portions of bladder catheters A and B. Urge incontinence attributed to catheter (A) was absent after spinalization.

Spinal Cord Transection, and Post-operative Care

The following section is quite detailed because this was the first time spinalization was performed for this contract.

Spinal cord transection at T10 level was carried out in two cats (Mick, Pascal). The procedure was performed under aseptic conditions in a fully-equipped operating theatre in the Health Sciences Facility of the University of Alberta. Assistance was provided by trained technicians (CALAS Certification). The cats were anesthetized with ketamine (intramuscular injection, 25 mg/kg) and intubated using an infant tracheal tube. Pre-op medication was administered: acepromazine 0.25 mg intramuscularly (i.m.), atropine 0.04 mg/kg i.m. and buprenorphine 0.01mg/kg subcutaneously (s.c). An intravenous (i.v.) catheter was inserted percutaneously into the cephalic vein and its hub was taped to the cat's forelimb and remained in place for 2-3 days, providing i.v. access for medications. The antibiotic Ampicillin was administered (250 mg i.v.).

The animal's back was shaved, washed with warm soap and water and scrubbed with iodine solution (betadine). Anesthesia was maintained with isoflurane (2% in carbogen, flow rate 1500 ml/min). A slow i.v. drip of sterile Ringers solution was administered to maintain fluid balance.

The skin was incised over the T10-L1 spinous processes and the paraspinal muscles were detached from the vertebrae. Rongeurs were used to create an opening at the T11-T12 vertebral junction. The dura mater was incised and a solution of 2% lidocaine (0.2 ml) was dripped on the surface of the cord. Two minutes later lidocaine was injected inside the spinal cord at progressively more ventral levels. Fine scissors were used to transect the cord. This delicate procedure was done using a high-resolution Leica dual-head operating microscope. The surgeon and assistant both verified the completeness of the transection by carefully inspecting both the rostral and caudal cut surfaces of the spinal cord through the slit in the dura. Surgicel, a blood coagulation promoter, was placed between the proximal and distal parts of the spinal cord. The dura was closed with two 8/0 silk ophthalmic sutures. The back incision was closed with 3-O Dexon® (braided polyglycolic acid suture, Davis & Geck) (muscle layer) and 3-O Novafil® (polybutester monofilament suture, Davis & Geck) (skin layer). At extubation, the cat was given Acepromazine, 0.25 mg and buprenorphine 0.01mg/kg s.c. and one or two small doses of pentobarbital (2mg/Kg) sufficient to maintain a somnolent state. During post-operative recovery the cats were kept warm in heated cages provided with blankets. Analgesia was maintained by giving two or three additional doses of buprenorphine at 8-hourly intervals. Ampicillin was administered for 4 days after surgery, followed by Amoxil (50 mg tablets, 2/day) for 6 additional days

Care of Animals following Spinal Cord Transection

After recovery the cats were housed in individual cages on soft pads. They received close nursing attention (monitoring hydration, body temperature, appetite, colo-rectal contents and defecation, bladder distension and urine leakage, incision sites, skin integrity), and intensive individual attention to help restore emotional and social equilibrium. They were spoken to, handled, groomed, assisted to sit and move and exercised with other cats for at least 1 hour a day in a large room set aside for this purpose. The room had a clean painted floor and was provided with blankets and toys. A wide variety of foods and treats were offered to encourage the resumption of normal feeding behavior. Cats had free access to water 24 hours/day and were fed moist and dry cat foods twice a day.

After low-thoracic spinal cord transection the bladder is areflexic for some time. The bladder was therefore drained twice daily through the implanted cannula, typically yielding 25-40 ml. Animal center staff kept a daily log of the cat's appetite and attitude, skin integrity, palpation of colo-rectal contents, bowel movements and any wetness from urinary leakage, as well as urine tests, therapies, perineal care/bathing. Urine tended to be cloudy for a few days post-operatively and then cleared. Research staff logged volume and time (minimum of 2x/day) of urinary drainage, the appearance of the drained urine, as well as bowel motions. Urine chemistry (multi-stick) was performed when the urine was cloudy or dark. Culture and sensitivity analysis was performed on two or three occasions so that appropriate antibiotics could be used to clear up low-grade urinary tract infections. The in-dwelling catheters implanted during this quarter performed well, both in regard to the twice-daily bladder voiding procedure and the measurement of bladder pressure changes evoked by ISMS.

Simultaneous intraurethral and intravesicular pressure measurements

In common with (McCreery, Lossinsky et al. 2002) we have adopted the method of (Brown and Wickham 1969) to measure intraurethral pressure. In this method, there is a slow, continuous infusion of sterile saline through the side-port of a catheter (in our case a Kendall Argyle 5 Fr. (1.7mm) feeding tube or a Kendall 3.5 Fr Tom Cat catheter), inserted about 25-30 mm into the urethra from the distal end (infusion rate 0.2 ml/min, Harvard Instruments Infusion Pump Model 22). This slightly inflates the urethra in the immediate vicinity of the side-port at the end of the catheter. Changes in tone in the urethral wall are then able to compress the saline in the inflated part of the urethra and within the catheter.

Pressure was measured at the far end of the catheter, close to the infusion pump, with a Neurolog NL108D4/10 dome and NL108T4 isolated pressure transducer. A second identical transducer system was used to measure intravesicular (bladder) pressure via the chronically implanted bladder catheter. The pressure signals were low-pass filtered at 30 Hz and sampled at 400 samples/s using a CED Power 1401 (Cambridge, UK) laboratory interface linked to a personal computer running CED Signal 2.1 software. The data were stored on the computer's hard drive for later analysis.

RESULTS

ISMS trials in implanted cats

Most of the experiments performed in this quarter were done in the two cats that underwent spinal transection (Mick and Pascal). Because the spinal transections were complete, sensory perception of urethral catheter insertion, intra-urethral stimulation and ISMS-evoked bladder and sphincter contractions was absent. Three sets of ISMS microwire implants failed for the following reasons.

1) Perry (implanted January 15). ISMS evoked increases in bladder pressure for 5 days. After this the microwires became non-functional. Dissection done on February 18 under pentobarbital anesthesia revealed that all the microwires had broken within their rubber tube between the L4 fixation point and the headpiece. We suspect that the tube became kinked and trapped under the skin and this led to excessive tension in the microwires during voluntary movements.

2) Pascal (implanted 27 January 2003). An error occurred during the implant: the wires were accidentally transected as the skin was being sutured. A repair was attempted on February 3. A full sterile procedure was performed in an HSLAS operating room using isoflurane anesthesia. The severed ends of the microwires were exposed through a skin incision and joined up by micro-soldering them to traces on a specially fabricated miniature circuit board. The board was then insulated with Dow Corning surface protector, RTV silastic, and buried subcutaneously. Although bladder responses were evoked during this procedure, by the next day and for the subsequent 2 weeks the only responses evoked were movements of the tail and perineal muscles. Pascal was subsequently spinalized and a terminal experiment was performed on 28 April to test

the hypothesis that the first neuronal structure to be excited by ISMS are afferent axons, not neuronal cell bodies. The results of this experiment will be reported in the next quarter.

3) Voodoo (implanted February 18 2003). To minimize the potential for microwire breakage, we implanted a back-pack connector in this case (see quarterly report #2 for design). The disposition of microwires in this implant is shown in Fig. 2. Good bladder contractions were evoked during the implant, but ISMS performed on February 24 evoked leg and tail movements but no bladder contractions from any of the microwires. The mode of failure of this implant is unknown. The cat is being held for chronic spinal transection in May 2003.

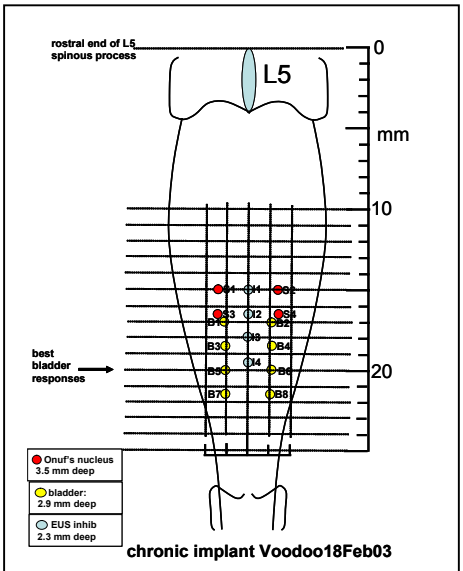

Figure 2 Microwire locations in Voodoo (implantedFeb18 2003).

Bladder pressures evoked by ISMS just before and just after complete spinal transection

Fig. 3 shows two ISMS trials in Mick, 6 months after the original implant (01 Oct 2002). The first trial occurred the day *before* spinalization and the second trial followed 2 days *after* spinalization. The same sequence of stimulation was used on the two occasions: three initial short bursts of stimulation through individual microwires (B1, B2 and S6: location shown in left part of the figure), followed by a short and a long burst of combined stimulation through all three microwires (interleaved pulses at a rate of 50/s per channel, 150/s combined rate). The short bursts via microwires B1 and B2 both elicited bladder pressure rises of 8-10mm Hg before and after spinalization. Microwire S6, even though it targeted the dorsal commissural region, evoked a larger increase in bladder pressure (about 15 mm Hg during the short burst). The long

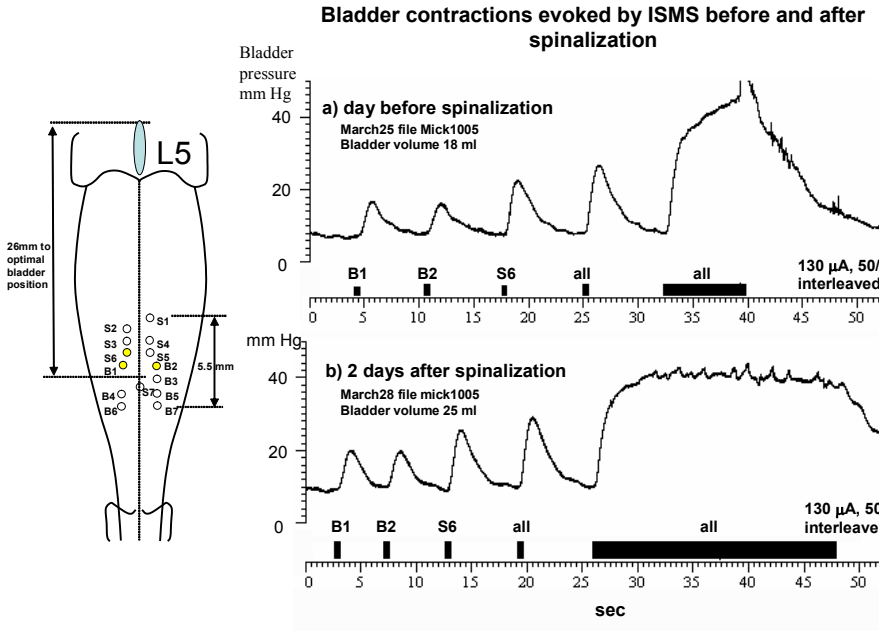

Figure 3. Bladder pressures evoked by ISMS before and after a complete spinal transection.

bursts of combined stimulation evoked sustained increases in bladder pressure of just over 40 mm Hg. There were no orienting or aversive responses to stimulation on either occasion.

The main significance of these results is the fact that ISMS evoked very similar bladder pressures before and after spinalization. In neither of the records shown in Fig. 3 did voiding occur, indicating that intra-urethral pressur exceeded bladder pressure throughout.

2. Stability of responses after spinalization .

Fig. 4 shows a trial performed 30 days after spinalization. The bladder pressures evoked by ISMS via microwires B1 and B2 were similar to those in the trials just before and just after spinalization shown in Fig. 1. However microwire S6 evoked a larger increase than it did just before and just after spinalization (~25mm Hg in Fig. 3 compared to ~15 mm Hg in Fig. 3). The intraspinal portion of the S1 microwire was 2.5 mm long, compared to 3.4 mm for B1 and B2. S6 was shorter because its tip was intended for the dorsal commisure, which has been implicated in the inhibition of motoneurons of the external urethral sphincter (Blok, van Maarseveen et al. 1998). The increase in the responses evoked by S6 30 days after spinalization is of some interest. It correlated with a general and very noticeable hyperreflexia of the hindlimbs that had gradually developed in this cat over the 30 days after spinalization. Exaggerated stretch reflexes, vigorous locomotor-like movements and paw-shake responses were readily evoked by minimal mechanical stimuli and wetting the paw by the time these data were collected.

Voiding was elicited in the trial of Fig. 4, but it was far from complete: 5 ml was voided, but the residual volume, confirmed by extraction through the cannula, was 15 ml. This contrasts with the complete voiding often elicited in this same cat by ISMS with identical parameters prior to spinalization. Certain features of the complete voiding responses prior to spinalization had suggested that a triggered reaction mediated by supraspinal centers was involved rather than a spinal reflex pathway (see previous quarterly report). The large residual volume in the trial in Fig. 4, and several similar trials not illustrated, lend further support to this suggestion.

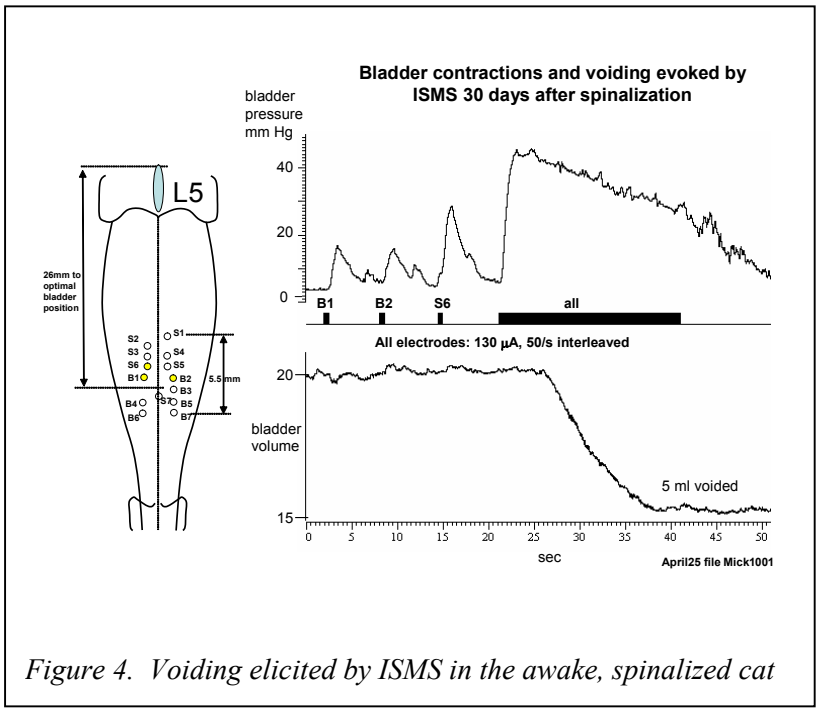

Figure 4. Voiding elicited by ISMS in the awake, spinalized cat

Intraurethral pressures.

Fig. 5 shows a trial in which bladder pressure and intraurethral pressure were simultaneously monitored in the awake, spinalized cat. The intraurethral pressure was measured with the use of a Kendall 3.5 Fr Tom Cat catheter, inserted about 30 mm into the urethra from the distal end (infusion rate 0.2 ml/min), using the technique of low-rate infusion described in Methods (Brown and Wickham 1969). In this trial, ISMS delivered via microwire S6 elicited increases in bladder pressure and simultaneous decreases in intraurethral pressure. Voiding could not occur because the catheter was a tight fit in the urethra, and blocked flow. However, during the combined stimulation burst, bladder pressure increased to 18 mm Hg and intraurethral pressure decreased to 22 mm Hg. If the bladder pressure had exceeded 22 mm as it did in the trials of Fig. 3 and 4, in theory there would have been a large enough difference in pressure to allow flow as in fact did occur in the trial illustrated in Fig. 4. The fact that the reduction in intraurethral pressure during the long burst in Fig. 5 was not sustained, may also explain why voiding ceased before stimulation was over in Fig. 4.

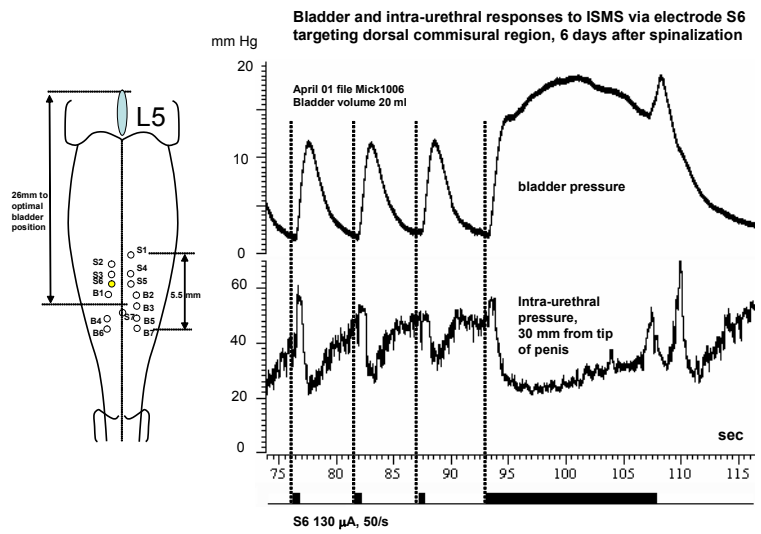

Figure 5 Intravesicular (bladder) pressure and intraurethral pressure simultaneously monitored in the awake, spinalized cat.

It should be noted however, that these inferences are speculative. There could be other reasons for the lack of complete voiding after spinalization in Figs. 3 and 4. The data were obtained on different days post-spinalization. The short bursts of stimulation via microwire S6 in the trial of Fig. 4 evoked an increase in bladder pressure of over 20 mm Hg, whereas in Fig. 5 a similar short burst only produced an increase of about 10 mm Hg. Intraurethral pressure may also have differed in the two trials. The slow infusion method elevates intraurethral pressure in a ramp-like manner until leakage occurs around the catheter and urine is voided. Intraurethral pressures ranged from 20 to 70 mm Hg in the trial of Fig. 5. Pressure within the urethra in the absence of infusion may be lower.

Though the data of Figs. 4 and 5 are encouraging with regard to the possibility of inhibiting urethral contractions, unfortunately the other 6 microwires targeting the dorsal commissural region did *not* produce inhibition.

For example, in the trial shown in Fig. 6, ISMS commenced with interleaved stimulation through B1, B2 and S6 and this was then supplemented for 3 sec with stimulation through electrodes S1, S2, S3, S4, and S5 (all pulses interleaved, 50/s each). The latter 5 microwires targeted the dorsal commissural region. The combined stimulation through all 8 microwires did not produce voiding. In fact, the rate of rise of bladder pressure clearly slowed when the dorsal commissural microwires were activated. Furthermore, when these microwires were tested individually, none elicited reductions in intraurethral pressure (this test was performed prior to spinalization in the isoflurane-anesthetized cat). Of course it remains to be seen post-mortem whether these microwires are indeed in the targeted locations.

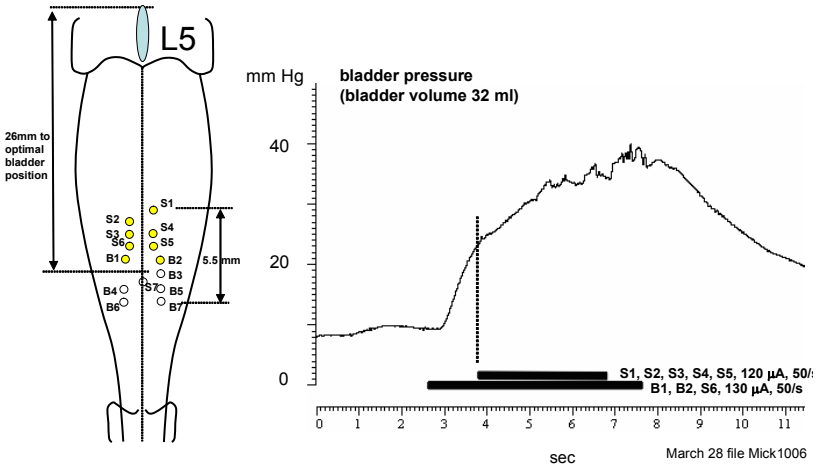

Figure 6. Bladder pressure response to ISMS via electrodes B1, B2 and S6, then combined with dorsal commisure electrodes S1-S5, 2 days after spinalization

DISCUSSION

The main development this quarter was the performance of the first two spinalization procedures and the comparison of responses evoked before and after spinalilzation. The main scientific findings were

1) that bladder pressure responses evoked by chronically implanted ISMS microwires were similar before and after spinalization.

2) Voiding was elicited by ISMS after spinalization, but it was not nearly as complete as before spinalization. This suggests that the pre-spinalization voiding may have involved a triggered supra-spinal component. It is quite possible that prior to spinalization, ISMS-evoked bladder contractions and the inhibitory action of microwire S6 on the urethra produced sensory perception which led to the urge to void. This would have been absent after complete spinalization. This could have an important clinical implication: in incomplete SCI people, ISMS might enhance the urge to void, and thus promote the micturition reflex.

3) ISMS via microwires targeting the dorsal commissural region did not in general elicit a reduction in intraurethral pressure, at least in the limited testing done so far. Microwire S6 was the one exception, and this electrode may have enabled the small amount of voiding we have so far been able to evoke post-spinalization.

PLANS FOR THE NEXT QUARTER

1) Further ISMS trials, including intraurethral pressure measurements in the remaining spinalized cat.

2) Two further chronic ISMS implants in Edmonton

- Characterize the types of bladder and sphincter responses elicited by multichannel ISMS in the sacral region in the awake animal, particularly in relation to bladder volume.
- Concentrate on eliciting urethral inhibition, either with ISMS or intra-urethral stimulation in combination with bladder contraction to elicit voiding.

ACKNOWLEDGEMENTS

We are grateful to Mr. Allen Denington for his valuable help in all aspects of this work. We are also most indebted to the staff of the Health Sciences Laboratory Animal Services Department of the University of Alberta for their conscientious nursing care and veterinary advice.

REFERENCES

Blok, B. F., J. T. van Maarseveen, et al. (1998). "Electrical stimulation of the sacral dorsal gray commissure evokes relaxation of the external urethral sphincter in the cat." Neuroscience Letters **249**(1): 68-70.

Brown, M. and J. E. A. Wickham (1969). " The urethral pressure profile." British Journal of Urology **41**: 211-217.

McCreery, D. B., A. Lossinsky, et al. (2002). Functional microstimulation of the lumbosacral spinal cord., NIH-NINDS contract # 1-NS-2-2340**:** 1-15.